ROBUST SUMMARY

PHYSICAL AND CHEMICAL DATA

1.0 MELTING POINT

Value:	136- 137°C
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Sublimation:	Yes [ ] No [X] Ambiguous [ ]
Method:	OECD Test Guideline 102 (1993)
GLP:	Yes [X] No [ ] ?[ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-1 from General Electric Company; Purity: 99.93%
Remarks:	The melting point (MP) of PI-01-1 was determined in duplicate as follows: The MP apparatus hot stage was heated to 120°C. PI-01-1 (approx. 0.5 mg) was placed on a glass slide that was placed on the hot stage of the MP apparatus and covered with a glass disc ('slip'). The hot stage was heated at less than 2°C/min, and the MP was recorded. Note that the MP is the range of temperatures from the temperature when PI-01-1 first started to melt, to the temperature at which all PI-01-1 in the field of view melted. It was also noted whether PI-01-1 changed color or appeared to decompose during the test. The MP of a calibration substance (phenanthrene) was similarly determined in duplicate. The observed duplicate melting point (MP) ranges for the reference compound (phenanthrene) were both 99 - 101°C, which compared to the expected MP of 100°C (Merck), indicating acceptable instrument calibration. The observed duplicate MP ranges of PI-01-1 were both 136 - 137°C. Therefore, the reported MP of PI-01-1 was 136 - 137°C. No color change or decomposition was observed during melting.
Reference:	Reimer, G.J. (2001). Unpublished report no. SP3241 1726-MP (BC Research Inc. Project no 3241 1726) entitled "Physical/chemical property of N-Methylphthalimide [CAS RN 550-44-7]: Melting Point (OECD 102)", dated August 9, 2001 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

2.0 BOILING POINT

Value:	275 ± 5°C
Pressure:	101.4 kPa
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Method:	OECD Test Guideline 103 (1993)
GLP:	Yes [X] No [ ] ?[ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-1 from General Electric Company; Purity: 99.93%
Remarks:	This analysis was conducted in duplicate, analyzing 2 separate pans containing PI-01-1. PI-01-1 (approx. 5 mg) was placed in an aluminum "pan" (3 mm OD) with a hole in the top of the pan (approx. 0.1 mm diam.). This pan was placed on the sample position of the differential

scanning calorimetry (DSC) instrument, and an empty pan was placed on the reference position. The thermocouple apparatus was warmed to approx. 320°C at 10°C/min. The differential heat flow was continuously recorded and plotted by the instrument. The instrument was calibrated (in duplicate) by boiling deionized water (approx. 5 mg), and by melting a sample of indium. The ambient air pressure during the experiment was 101.4 kPa. DSC Calibration: the DSC instrument showed an average bias of 2.45°C for the boiling of water, and a bias of 0.19°C for the melting of indium. The DSC results on PI-01-1 were corrected for the bias of 2.45°C. PI-01-1 Analysis: the average boiling point of PI-01-1 was 275 ± 5°C (RSD = 2%) at 101.4 kPa.

Reference: Reimer, G.J. (2001). Unpublished report no. SP3241 1726-BP (BC Research Inc. Project no. 3241 1726) entitled "Physical/chemical property of N-Methylphthalimide [CAS RN 550-44-7]: Boiling Point (OECD 103)", dated August 9, 2001 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

3.0 VAPOR PRESSURE

Value: 0.000513 HPa

Temperature: 24.15°C

Method: Calculated [ ] Measured [X]
OECD Test Guideline 104 (1993) – Thermogravimetric Analysis with a Knudsen Cell.

GLP: Yes [ ] No [ ] ? [X]

Test Substance: N-Methylphthalimide (PI, CAS RN 550-44-7); prepared by condensation of phthalic anhydride with aqueous methylamine. The product, obtained in 91% yield, was recrystallized and confirmed by NMR (final purity not stated).

Remarks: Vapor pressures of PI-01-1 as a function of temperature were reported by Roux (1997), using a Thermogravimetric Analysis (TGA) method with a Knudsen cell. The reported vapor pressure of PI-01-1 at 24.15°C was 0.0513 Pa. Other reported vapor pressures at higher temperatures are reproduced in the following table:

Temperature		Vapor Pressure			
°C	1/°C	Torr (mm Hg)	Log(torr)	Pascal	Log(pascal)
25.15	0.03976	0.000385	-3.415	0.0513	-1.290
28.23	0.03542	0.000548	-3.262	0.0730	-1.137
30.07	0.03326	0.000689	-3.162	0.0919	-1.037
34.11	0.02932	0.001103	-2.958	0.1470	-0.833
37.27	0.02683	0.001575	-2.803	0.2100	-0.678
40.15	0.02491	0.002175	-2.663	0.2900	-0.538
43.11	0.02320	0.003075	-2.512	0.4100	-0.387

Reference: Reimer, G.J. (2001). Unpublished report no. SP3241 1726-VP (BC Research Inc. Project no. 3241 1726) entitled "Physical/chemical property of N-Methylphthalimide [CAS RN 550-44-7]: Vapor Pressure", dated August 9, 2001 for General Electric Company,

Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada. This report reviews the results from the following publication:

Roux, Maria Victoria; Jimenez, Pilar; Martin-Luengo, Maria Angeles; Davalos, Juan Z.; Sun, Zhiyuan; Hosmane, Ramachandra S.; Liebman, Joel F. The Elusive Antiaromaticity of Maleimides and Maleic Anhydride: Enthalpies of Formation of N-Methylmaleimide, N-Methylsuccinimide, N-Methylphthalimide, and N-Benzoyl-N-methylbenzamide. J. Org. Chem. (1997), 62(9), 2732-2737.

Reliability: (Klimisch Code 2) Valid with restrictions. Results from a well-documented publication.

4.0 PARTITION COEFFICIENT ($Log_{10}P_{OW}$)

Log Pow:	19 ± 2
Temperature:	22 ± 1ºC
Method:	Calculated [ ] Measured [X] Based on OECD Test Guideline 107 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-1 from General Electric Company; Purity: 99.93%
Remarks:	Test Water: N-Octanol (20 mL) was added to 1 L of deionized water. This mixture was shaken vigorously for 1 min, and allowed to settle before use. Test Octanol: Deionized water (20 mL) was added to N-octanol (1 L). This mixture was shaken vigorously for 1 min, and allowed to settle before use. SkPI6 (Stock solution of PI-01-1 in Test Water): PI-01-1 (11.60 mg; 5-place electronic balance) was dissolved in Test Water solution (100.00 mL; 0.1160 mg/mL). SkPI7 (isopropanol stock solution of PI-01-1): PI-01-1 (10.83 mg) was dissolved in isopropanol (IPA) solution (25.00 mL; 0.4332 mg/mL) in a volumetric flask; solution stored at 4 °C in a sealed vial. IPA Calibration Solutions: SkPI7 (1.50 mL; pipettor) was serially diluted 5-times into IPA (1.50 mL) for calibration standards ranging from 13.5 to 216.6 µg/mL. These solutions were transferred to 1.8-mL glass crimp-top vials and stored at -20°C. Before use, they were warmed to > 10 °C and mixed. Added into ten 15-mL polypropylene centrifuge tubes were aqueous PI-01-1 solution SkPI6 (5.00 mL; pipettor), and Test Octanol (pipettor or pipette). Test Octanol and Test Water were added to the blank tube. The contents of each tube were mixed (rotation about tube's horizontal axis at approx. 1 rev/sec) for 30 min. The mixing start and stop times and air temperature were recorded. After settling, the Octanol Test Phases (approx. 1.5 mL) were aliquoted onto 1.8-mL glass crimp-top vials. The remaining octanol layers were aspirated (discarded) from the tubes. The Aqueous Test Phases (approx. 1.5 mL) were aliquoted onto 1.8-mL glass crimp-top vials. Care was taken not to include any octanol phase (which could have a relatively high PI-01-1 concentration). This was done by lowering a Pasteur pipette tip into the aqueous phase while slowly expelling air from the pipette. The

pipette was removed and the first few drops discarded. Part of the pipette volume was transferred to the 1.8-mL glass crimp-top vial and a small volume of aqueous phase was left in the pipette. The pipette was discarded. The ambient air temperature during the test was 22 ± 1°C.

The concentrations (C_{aq}) of PI-01-1 in Aqueous Test Solutions were calculated as follows: $C_{aq} = (S-b) \div m$; where *S* was the HPLC peak area of the PI-01-1 peak, and *m* and *b* were the slope and y-intercept respectively of the calibration curve (from HPLC-DAD results of Aqueous Calibration Solutions). The concentrations (C_{oct}) of PI-01-1 in Octanol Test Solutions were calculated as follows: $C_{oct} = ((S-b) \div m)F$; where *S* was the GC-FID peak area of the PI-01-1 peak, and *m* and *b* were the slope and y-intercept respectively of the calibration curve (from GC-FID results of IPA Calibration Solutions). Because the solvent of the IPA Calibration Solutions (isopropanol) was different from that of the Octanol Test Solutions (octanol), any effect that octanol had on the response of PI-01-1 was accounted for by using the factor F in the above equation. F (1.207) was calculated as follows: $F = C_{exp} \div C_{obs}$; where: C_{exp} = Expected PI-01-1 concentration in solution GCME (216.6 µg/L) C_{obs} = Observed PI-01-1 concentration in solution GCME (179.4 µg/L). The octanol/water partition coefficient (P_{ow}) was calculated as follows: $P_{ow} = C_{oc\,t} \div C_{aq}$; where C_{oct} and C_{aq} were the concentrations of the test substance in the octanol and aqueous phases respectively. Mass balance (M) was calculated using the following equation: $M = Q_{oct} + Q_{aq} \div Q_{added}$; where Q_{oct} and Q_{aq} were the observed masses of PI-01-1 in the Octanol Test Phase and Aqueous Test Phase respectively, and Q_{added} was the initial mass of PI-01-1 added to the test tube.

The calibration curves from the IPA Calibration Solutions (for octanol phases) and Aqueous Calibration Solutions showed linear relationships with correlation coefficients (R^2) of 0.998 and 0.999 respectively, indicating acceptable method precision for the analysis of PI-01-1 in these solutions. The detection limit may be defined as the 'lowest concentration of an analyte that an analytical process can reliably detect' (Long *et al.*, 1983). The Instrument Detection Limit (IDL) was qualitatively estimated at 3 µg/mL (corresponding to 3 ng of PI-01-1 injected on-column) for the detection of PI-01-1 in IPA solution. The Instrument Detection Limit (IDL) was qualitatively estimated at 2 µg/mL (corresponding to 8 ng of PI-01-1 injected on-column) for the detection of PI-01-1 in aqueous solution. The average percent relative (population) standard deviations (%RSD) of the observed duplicate concentrations of PI-01-1 in the octanol and aqueous Test Phases were 3% and 2% respectively, indicating acceptable intra-assay method precision for the analysis of PI-01-1 in octanol and aqueous solutions.

As determined in a water solubility test, the pH of a PI-01-1-saturated aqueous solution was 4.5, and the pH of the 'blank' (pure) water was 5.5. PI-01-1 was observed in the Octanol and Aqueous Test Phases.

The average mass balance was 85 ± 3%. The average observed P_{ow} was 19 ± 2 ($\log_{10}(P_{ow})$ = 1.29 ± 0.04) at the test temperature of 22 ± 1°C.

Reference: Reimer, G.J. (2001). Unpublished report no. SP3241 1726-BP (BC Research Inc. Project no. 3241 1726) entitled "Physical/chemical property of N-Methylphthalimide [CAS RN 550-44-7]: Octanol/Water Partition Coefficient (OECD 107)", dated August 9, 2001 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

5.0 WATER SOLUBILITY

5.1. SOLUBILITY

Value: 420 ± 50 mg/L
Temperature: 22.6 ± 0.3°C
Description: Miscible [ ]; Of very high solubility [ ]; Of high solubility [ ]; Soluble [ ]; Slightly soluble [X]; Of low solubility [ ]; Of very low solubility [ ]; Not soluble [ ]
Method: OECD Test Guideline 105 (1993)
GLP: Yes [X] No [ ] ? [ ]
Test Substance: N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-1 from General Electric Company; Purity: 99.93%
Remarks: Powdered PI-01-1: PI-01-1 (approx. 3 g) was ground to a fine powder in a clean (washed with water and acetone or MeOH, and thoroughly dried) mortar and pestle. Stock Solution SkPI1: PI-01-1 (5.78 mg) was dissolved in 5.00 mL of CH_3CN solution in a volumetric flask (1.156 mg/mL), and stored in capped glass vial in a refrigerator. Calibration Solutions: Stock Solution SkPI1 (1.00 mL) was serially diluted 6-times into CH_3CN (1.00 mL), in single-use 5-mL glass test tubes, to give solutions ranging from 18.1 to 578 μg/mL. The solutions were transferred to 1.8-mL glass crimp-top vials.

Saturated Solutions: The powdered PI-01-1 was added to 15-mL centrifuge tubes except the blank (blk). Deionized water (5.0 mL) was added to each tube. The contents of all tubes were mixed (tumbled end-over-end; approx. 20 rpm) at 22.6 ± 0.3°C for 3.0 days or 4.0 days. After mixing for 3 days, the PW3 solutions were centrifuged (3000 rpm, 5 min.), and sat on the bench. Similarly, the PW4 samples were centrifuged after mixing for 4 days. It was noted whether undissolved PI-01-1 was present in all tubes (except blank). The pH of solution PW4-c and the blank water (PW4-blk) were measured (pH strip). Each solution (including blank; approx. 1.5 mL) was filtered (0.45 μm plastic syringe filter) into 1.8-mL glass crimp-top vials. These clear filtered Test Solutions were diluted in deionized water as follows: Aliquots (100 μL; pipettor) of these filtered solutions were transferred to another set of 1.8-mL glass crimp-top vials, all of which contained 1.00 mL deionized water.

The concentrations (*C,* μg/mL) of PI-01-1 in diluted Test Solutions

were calculated as follows: C = (S-b) ÷ m(df); where *S* was the peak area of the PI-01-1 peak, *m* and *b* were the slope and y-intercept respectively of the calibration curve, and *df* was the Test Solution dilution factor (0.090909091).

The calibration curve from the Calibration Solutions showed a linear relationship with a correlation coefficient of 0.9998, indicating acceptable method precision for the analysis of PI-01-1 in acetonitrile solution. The detection limit was defined as the lowest concentration of an analyte that an analytical process can reliably detect. The Instrument Detection Limit (IDL) was qualitatively estimated at 1 μg/mL (corresponding to 5 ng of PI-01-1 injected on-column) for the detection of PI-01-1 in acetonitrile solution, based on the chromatograms of Calibration Solutions.

After mixing for 3 or 4 days, undissolved PI-01-1 was observed in all (non-blank) test tubes. The pH of one of the Test Solutions (PW4-c) after mixing was 4.5, and the pH of the blank water (PW4d-blk) after mixing was 5.5. This peak presumably corresponded to N-methylphthalimide. There was no interfering peak in the water blank. After mixing for 3 and 4 days, the observed PI-01-1 concentrations in the Test Solutions (350 ± 90 μg/mL and 420 ± 50 μg/mL) were not significantly different, and the latter value (420 μg/mL) was 17% higher than the former (350 μg/mL) that was similar to the OECD-recommended maximum difference of 15%. From these results it was concluded that saturation of the Test Solutions was achieved after 4 days of mixing. The reason for the relatively high %RSD value for the 'PW3d' average PI-01-1 concentration (26%) was not known, but may have been due to variability in the filtration step, possibly due to the formation of N-methylphthalimide micelles. These 'PW3d' results were used only to demonstrate that saturation occurred. Therefore, the average observed water solubility of PI-01-1 was 420 ± 50 μg/mL (mg/L) at 22.6 ± 0.3 °C. This value was the average of the PI-01-1 concentrations in the 'PW4d' Test Solutions.

Reference: Reimer, G.J. (2001). Unpublished report no. SP3241 1726-WS (BC Research Inc. Project no. 3241 1726) entitled "Physical/chemical property of N-Methylphthalimide [CAS RN 550-44-7]: Solubility in Water (OECD 105)", dated August 9, 2001 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

5.2. pH VALUE, pKa VALUE

No studies were found.

ENVIRONMENTAL FATE AND PATHWAYS

6.0 PHOTODEGRADATION

Synopsis of a report entitled* "Fugacity Modeling to Estimate Transport Between Environmental Compartments for N-Methylphthalimide (PI) (CAS Reg. No. 550-44-7), *dated November 10, 2001 for General Electric Company, Pittsfield, MA, USA from Charles A. Staples, Ph.D., Assessment Technologies, Inc. Fairfax, VA, USA.

Reported here are the results of fugacity-based distribution modeling conducted for N-methylphthalimide (PI). A number of study reports containing data needed for the modeling were provided and used. This document contains a brief synopsis of physical property and environmental fate property estimation, fugacity-based distribution modeling, modeling results for PI, plus a few comments on the studies that were used.

Fugacity-based Distribution Modeling

Introduction

Fugacity-based distribution modeling requires several physical properties and fate characteristics as model inputs. Property estimation programs were used to obtain estimates of any physical property or fate characteristic (e.g., atmospheric photo-oxidation and biodegradation) for which data were not provided. To estimate the physical properties and fate characteristics, several models can be employed. The models were based on structure-activity relationships (SAR) and were used to estimate hydroxyl radical mediated atmospheric photo-oxidation for PI.

Estimation of Physical Properties

The SAR models for estimating physical properties and abiotic degradation were developed by the U.S. Environmental Protection Agency and Syracuse Research Corporation (Estimation Programs Interface for Windows, Version 3.05 or EPIWIN v.3.05) (Syracuse 2000). The models can be used to calculate melting point, vapor pressure (submodel MPBPVP), octanol-water partition coefficient or Kow (submodel KOWWIN), and aqueous solubility (submodel WSKOWWIN). The calculation procedures are described in the program guidance and are adapted from standard procedures based on analysis of key structural features (Meylan and Howard, 1999a,b,c). Key assumptions and default parameters used in the models were developed under U.S. EPA guidance. EPA uses the models for various regulatory activities.

Estimation of Environmental Fate

Atmospheric photo-oxidation potential was estimated using the submodel AOPWIN (Meylan and Howard, 2000). The estimation methods employed by AOPWIN are based on the SAR methods developed by Dr. Roger Atkinson and co-workers (Meylan and Howard, 2000a). The SAR methods rely on structural features of the subject chemical. The model calculates a second-order half-life with units of cm^3/molecules-sec. Photo-degradation based on atmospheric photo-oxidation is in turn based on the second order rate of reaction (cm^3/molecules-sec) with hydroxyl radicals (HO•), assuming first-order kinetics and an HO• concentration of 1.5 E+6 molecules/cm^3 and 12 hours of daylight. Pseudo-first order half-lives ($t_{1/2}$) were then calculated as follows: $t_{1/2} = 0.693 / [k_{phot} \times HO\bullet \times 12\text{-hr} / 24\text{-hr}]$.

Estimation of Environmental Distributions

The fugacity-based distribution model was based on the Trent University Modeling Center's EQuilibrium Concentration model (EQC) Level 3 model, version 1.01. These models are described in Mackay et al. (1996a,b). Fugacity-based modeling is based on the "escaping"

tendencies of chemicals from one phase to another. For instance, a Henry's Law constant calculated from aqueous solubility and vapor pressure is used to describe the "escape" of a chemical from water to air or vice versa as it seeks to attain equilibrium between the phases. The key physical properties required as input parameters into the model are melting point, vapor pressure, octanol-water partition coefficient (Kow), and aqueous solubility. The model also requires estimates of first-order half-lives in air, water, soil, and sediment. An additional key input parameter is loading or emissions of the chemical into the environment. The default assumption was used here, which assumes equal releases to air, water, and soil. The model was run using the chemical specific parameters to obtain estimates of the chemical distributions between environmental compartments.

Common Features of the Models

All of the models use the structure of the molecule to begin performing the calculation. The structure must be entered into the model in the form of a SMILES notation or string (Simplified Molecular Input Line Entry System). It is a chemical notation system used to represent a molecular structure by a linear string of symbols. The SMILES string allows the program to identify the presence or absence of various structural features that control aspects of the submodels. The models do contain structures and SMILES strings for about 100,000 compounds, accessible via Chemical Abstracts Service (CAS) Registry number.

Model Results

Based on the reports provided to me, the following measured data were reported and used in the fugacity-based distribution modeling. PI undergo hydrolysis that is strongly pH dependent with half-lives of >10,000 hours at pH 4, 88 hours at pH 7, and 2.2 hours at pH 9 (all at 25°C) (Reimer, 2001a), has a melting point averaging 136.5°C (Reimer, 2001b), a vapor pressure of 0.0513 Pa (Reimer, 2001c), a log Kow value of 1.29 (Reimer, 2001d), and a water solubility of 420 mg/L (22.6°C) (Reimer, 2001e). Using the EPIWIN models described above, additional parameters were estimated. They include an atmospheric half-life of 15.7 hours, water and soil biodegradation half-lives of 360 hours, and a sediment half-life of 1440 hours. Excepting the water and soil biodegradation half-lives, these values were all used in the distribution modeling. Hydrolysis is the dominant fate process in water and would be in soil also, so an 88 hour half-life (assuming an environmental pH value of 7.0) was used in those compartments. The results of the distribution modeling (assuming equal emissions to air, water, and soil) were: Air 5.3%, Water 44.4%, Soil 50.2%, and Sediment <0.1%.

One of the PI reports dealt with measured soil adsorption, reporting soil partition coefficients (Koc values) (Reimer, 2001f). The technique was to stir soil and water containing test chemical for 16 hours. At experiments end, the amount of PI remaining in solution was measured. The study used two soils with pH values of 5.1 and 5.7 and a third soil with pH 9. The organic carbon normalized Koc values for the two soils at pH 5.1 and 5.7 were 140 and 548 L/kg. The values are plausible given the log Kow value of 1.29. The Koc value for the third soil was reported to be 2636 L/kg. This was based on the absence of detectable PI in solution and the assumption that all PI was, therefore, sorbed onto the soil. This is not a correct interpretation of the results. A hydrolysis half-life of 2.2 hours was measured at pH 9. The soil solution was at pH 9. More than 7 half-lives would occur during the 16 hour test. The amount of PI in the system at the end of the test would be <0.78% of the initial test substance. PI would not accumulate in or sorb onto soil at pH values in the range of 9. It would instead rapidly hydrolyze. The reported Koc value for the third soil cannot be considered correct.

References

Mackay, D. et al. 1996a. Assessing the fate of new and existing chemicals: a five-stage process. Environ. Toxicol. Chem. 15(9): 1618-1626.

Mackay,D. et al. 1996b. Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Environ. Toxicol. Chem. 15(9): 1627-1637.
Meylan, W. and PH Howard. 1999a. User's Guide for MPBPVP, Version 1.4. Syracuse Research Corporation. North Syracuse, New York. December, 1999.
Meylan, W. and PH Howard. 1999b. User's Guide for KOWWIN, Version 1.6. Syracuse Research Corporation. North Syracuse, New York. July, 1999.
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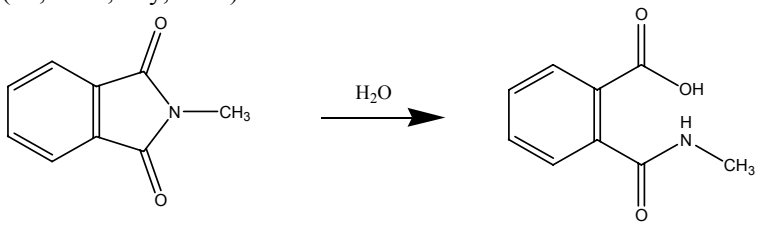
Meylan, W. and PH Howard. 2000a. User's Guide for AOPWIN, Version 1.9. Syracuse Research Corporation. North Syracuse, New York. March, 2000.
Meylan, W. and PH Howard. 2000b. User's Guide for BIOWIN, Version 4.0. Syracuse Research Corporation. North Syracuse, New York. February, 2000.
Reimer Analytical & Associates, Inc. 2001a. Physical-Chemical Property of TNPP (26523-78-4). Hydrolysis as a Function of pH (OECD 111). Study No. 171-1234HY, Vancouver, BC, Canada, August 10, 2001.
Reimer Analytical & Associates, Inc. 2001b. Physical-Chemical Property of TNPP (26523-78-4). Melting Point (OECD 102). Study No. 171-1234MP, Vancouver, BC, Canada, August 10, 2001.
Reimer Analytical & Associates, Inc. 2001c. Physical-Chemical Property of TNPP (26523-78-4). Solubility in Water (OECD 105). Study No. 171-1234SW, Vancouver, BC, Canada, August 10, 2001.
Reimer Analytical & Associates, Inc. 2001d. Physical-Chemical Property of TNPP (26523-78-4). N-Octanol/Water Partition Coefficient (Expert Statement) Study No. 171-1234, Vancouver, BC, Canada, August 10, 2001.
Syracuse Research Corporation. 2000. User's Guide for Estimation Programs Interface for Windows, Version 3, Syracuse Research Corporation. North Syracuse, New York.

7.0 STABILITY IN WATER

Type:	Abiotic (hydrolysis) [X]; biotic (sediment) [ ]
Half life:	10148, 88, and 2.2, hours at 25°C at pH 4, pH 7, and pH 9, respectively
Degradation:	Yes (See remarks below)
Method:	OECD Test Guideline 111 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-1 from General Electric Company; Purity: 99.93%
Remarks:	The hydrolysis of PI-01-1 results in phthalimide ring opening to give the N-methylphthalamic acid hydrolysis product as shown below (Su, 1969; Iley, 1998).



pH 4 Buffer: An acetate buffer was prepared by dissolving 1.16 mL of glacial acetic acid in 1.00 L of deionized water solution (solution A; 0.02 M). Solution B was prepared by dissolving 1.64 g of anhydrous sodium acetate (or 2.72 g of the trihydrate) in 1.00 L of deionized

water solution (0.02 M). Solution A (41.0 mL) and Solution B (9.0 mL) were mixed. The pH of this buffer was confirmed with a pH strip. pH 7 Buffer: MOPS free acid (20.93 g; 0.05 M) was dissolved in 2.0 L of deionized water solution. The solution was titrated to pH 7.0 with approx. 70 mL of 0.1 N aqueous NaOH. pH 9 Buffer: Dissolved 1.22 g of ethanolamine (0.02 M) in approx. 900 mL of deionized water. Titrated to pH 9.0 with monovalent strong base or acid as needed. The solution was made up volume to 1000 mL with deionized water.

HYDROLYSIS AT 22ºC: PI-01-1 solution in CH_3CN (SkPI6): PI-01-1 (18.48 mg) was dissolved in 25.00 mL of CH_3CN solution in a volumetric flask (0.7392 mg/mL; 0.004586 M). Diluted solution SkPI6D: SkPI6 PI-01-1 solution (100 µL; pipettor) was mixed with CH_3CN (1.50 mL) in a 1.8-mL glass crimp-top vial (46.2 µg/mL). The following was done rapidly, to minimize hydrolysis before HPLC analyses. SkPI6 PI-01-1 solution (100 µL; pipettor) was added to the respective vials. The time of this addition was recorded. These Buffer Solutions in the capped vials were mixed and analyzed by HPLC. The test temperature was 22 ± 1°C.

HYDROLYSIS AT 50ºC: 1.8-mL glass screw-cap vials with were used. The appropriate buffers (1.50 mL; pipettor) were added to the vials and SkPI6 or CH_3CN (100 µL; pipettor) were then added to the vials. (dil. fact. = 0.06250; PI-01-1 concentration = 46.2 µg/mL; 0.2866 mM). The pHs of the three time-zero vials were measured with pH strips. The vials were capped (teflon-lined septa) and the solutions were mixed. The caps were tightly secured to prevent evaporation during 5 days at 50 °C. The three time-zero vials were frozen at –20 °C. The remaining vials were placed in a GC oven maintained at 50.3 ± 0.1 °C. At intervals, the vials were removed and frozen at -20 °C. On the 5th day, the pHs of the three 17-hour samples were measured with pH strips before placing them at -20 °C.

The calibration curve from the Calibration Solutions showed a linear relationship with a correlation coefficient (R^2) of 0.9997, indicating acceptable method precision for the analysis of PI-01-1 in acetonitrile solution. The detection limit was defined as the lowest concentration of an analyte that an analytical process can reliably detect. The Instrument Detection Limit (IDL) was qualitatively estimated at 0.4 µg/mL (corresponding to 1.6 ng of PI-01-1 injected on-column) for the detection of PI-01-1 in acetonitrile solution, based on the chromatograms of Calibration Solutions.

The concentrations of the Test Substance N-methylphthalimide (PI-01-1) in aqueous pH 4, pH 7, and pH 9 buffer solutions were monitored over time. The hydrolysis of PI-01-1 was relatively rapid at pH 9, permitting the use of ambient temperature (22 ± 1 °C) in this experiment. A warmer temperature (50°C) was required at pH 4 and pH 7 due to the slower hydrolysis rates at these pH's. The observed hydrolysis rate constants and half-lives of PI-01-1 are summarized in the Table below. The OECD defines a hydrolytically stable

compound as one with a half-life greater than one year (> 8,760 h; OECD 111) at 25°C. PI-01-1 was observed to be 'unstable' at pH 7 and pH 9 at 22°C, because the PI-01-1 half-lives ($t_{1/2}$) were much less than 8,760 h under these conditions. At pH 4, the observed PI-01-1 half-life of 10,148 h at 25°C was 14% greater than the OECD criterion of 8,760 h. Given that surrogate data were used in the estimation of this PI-01-1 half-life (pH 4, 25°C), these half-lives were not considered to be significantly different, and it was conservatively concluded that PI-01-1 would also be defined as 'unstable' at pH 4 and 25°C.

Kinetics results for PI-01-1 hydrolysis:

	25°C			50°C		
pH	**Rate Constant**	**Units**	**t1/2 (hr)**	**Rate Constant**	**Units**	**t1/2 (hr)**
4	4.1E-12	M sec^{-1}	10,148	5.51E-11	M sec^{-1}	762
7	2.2E-06	sec^{-1}	88	2.91E-05	sec^{-1}	6.6
9	8.7E-05	sec^{-1}	2.2	nd		nd

Reference: Reimer, G.J. (2001). Unpublished report no. SP3241 1726-Hy (BC Research Inc. Project no. 3241 1726) entitled "Physical/chemical property of N-Methylphthalimide [CAS RN 550-44-7]: Hydrolytic Stability (OECD 111)", dated August 9, 2001 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Iley, Jim; Calheiros, Teresa; Moreira, Rui, Phthalimidomethyl as a drug pro-moiety. Probing its reactivity. Bioorg. Med. Chem. Lett. (1998), 8(8), 955-958.

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Reliability: (Klimisch Code 1) Valid without restrictions.

8.0 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS, INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

8.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Synopsis of a report entitled* "Fugacity Modeling to Estimate Transport Between Environmental Compartments for N-Methylphthalimide (PI) (CAS Reg. No. 550-44-7), *dated November 10, 2001 for General Electric Company, Pittsfield, MA, USA from Charles A. Staples, Ph.D., Assessment Technologies, Inc. Fairfax, VA, USA.

Reported here are the results of fugacity-based distribution modeling conducted for N-methylphthalimide (PI). A number of study reports containing data needed for the modeling were provided and used. This document contains a brief synopsis of physical property and environmental fate property estimation, fugacity-based distribution modeling, modeling results for PI, plus a few comments on the studies that were used.

Fugacity-based Distribution Modeling

Introduction

Fugacity-based distribution modeling requires several physical properties and fate characteristics as model inputs. Property estimation programs were used to obtain estimates of any physical property or fate characteristic (e.g., atmospheric photo-oxidation and biodegradation) for which data were not provided. To estimate the physical properties and fate characteristics, several models can be employed. The models were based on structure-activity relationships (SAR) and were used to estimate hydroxyl radical mediated atmospheric photo-oxidation for PI.

Estimation of Physical Properties

The SAR models for estimating physical properties and abiotic degradation were developed by the U.S. Environmental Protection Agency and Syracuse Research Corporation (Estimation Programs Interface for Windows, Version 3.05 or EPIWIN v.3.05) (Syracuse 2000). The models can be used to calculate melting point, vapor pressure (submodel MPBPVP), octanol-water partition coefficient or Kow (submodel KOWWIN), and aqueous solubility (submodel WSKOWWIN). The calculation procedures are described in the program guidance and are adapted from standard procedures based on analysis of key structural features (Meylan and Howard, 1999a,b,c). Key assumptions and default parameters used in the models were developed under U.S. EPA guidance. EPA uses the models for various regulatory activities.

Estimation of Environmental Fate

Atmospheric photo-oxidation potential was estimated using the submodel AOPWIN (Meylan and Howard, 2000). The estimation methods employed by AOPWIN are based on the SAR methods developed by Dr. Roger Atkinson and co-workers (Meylan and Howard, 2000a). The SAR methods rely on structural features of the subject chemical. The model calculates a second-order half-life with units of cm^3/molecules-sec. Photo-degradation based on atmospheric photo-oxidation is in turn based on the second order rate of reaction (cm^3/molecules-sec) with hydroxyl radicals (HO•), assuming first-order kinetics and an HO• concentration of 1.5 E+6 molecules/cm^3 and 12 hours of daylight. Pseudo-first order half-lives ($t_{1/2}$) were then calculated as follows: $t_{1/2} = 0.693 / [k_{phot} \times HO\bullet \times 12\text{-hr} / 24\text{-hr}]$.

Estimation of Environmental Distributions

The fugacity-based distribution model was based on the Trent University Modeling Center's EQuilibrium Concentration model (EQC) Level 3 model, version 1.01. These models are described in Mackay et al. (1996a,b). Fugacity-based modeling is based on the "escaping" tendencies of chemicals from one phase to another. For instance, a Henry's Law constant calculated from aqueous solubility and vapor pressure is used to describe the "escape" of a chemical from water to air or vice versa as it seeks to attain equilibrium between the phases. The key physical properties required as input parameters into the model are melting point, vapor pressure, octanol-water partition coefficient (Kow), and aqueous solubility. The model also requires estimates of first-order half-lives in air, water, soil, and sediment. An additional key input parameter is loading or emissions of the chemical into the environment. The default assumption was used here, which assumes equal releases to air, water, and soil. The model was run using the chemical specific parameters to obtain estimates of the chemical distributions between environmental compartments.

Common Features of the Models

All of the models use the structure of the molecule to begin performing the calculation. The structure must be entered into the model in the form of a SMILES notation or string (Simplified Molecular Input Line Entry System). It is a chemical notation system used to

represent a molecular structure by a linear string of symbols. The SMILES string allows the program to identify the presence or absence of various structural features that control aspects of the submodels. The models do contain structures and SMILES strings for about 100,000 compounds, accessible via Chemical Abstracts Service (CAS) Registry number.

Model Results

Based on the reports provided to me, the following measured data were reported and used in the fugacity-based distribution modeling. PI undergo hydrolysis that is strongly pH dependent with half-lives of >10,000 hours at pH 4, 88 hours at pH 7, and 2.2 hours at pH 9 (all at 25°C) (Reimer, 2001a), has a melting point averaging 136.5°C (Reimer, 2001b), a vapor pressure of 0.0513 Pa (Reimer, 2001c), a log Kow value of 1.29 (Reimer, 2001d), and a water solubility of 420 mg/L (22.6°C) (Reimer, 2001e). Using the EPIWIN models described above, additional parameters were estimated. They include an atmospheric half-life of 15.7 hours, water and soil biodegradation half-lives of 360 hours, and a sediment half-life of 1440 hours. Excepting the water and soil biodegradation half-lives, these values were all used in the distribution modeling. Hydrolysis is the dominant fate process in water and would be in soil also, so an 88 hour half-life (assuming an environmental pH value of 7.0) was used in those compartments. The results of the distribution modeling (assuming equal emissions to air, water, and soil) were: Air 5.3%, Water 44.4%, Soil 50.2%, and Sediment <0.1%.

One of the PI reports dealt with measured soil adsorption, reporting soil partition coefficients (Koc values) (Reimer, 2001f). The technique was to stir soil and water containing test chemical for 16 hours. At experiments end, the amount of PI remaining in solution was measured. The study used two soils with pH values of 5.1 and 5.7 and a third soil with pH 9. The organic carbon normalized Koc values for the two soils at pH 5.1 and 5.7 were 140 and 548 L/kg. The values are plausible given the log Kow value of 1.29. The Koc value for the third soil was reported to be 2636 L/kg. This was based on the absence of detectable PI in solution and the assumption that all PI was, therefore, sorbed onto the soil. This is not a correct interpretation of the results. A hydrolysis half-life of 2.2 hours was measured at pH 9. The soil solution was at pH 9. More than 7 half-lives would occur during the 16 hour test. The amount of PI in the system at the end of the test would be <0.78% of the initial test substance. PI would not accumulate in or sorb onto soil at pH values in the range of 9. It would instead rapidly hydrolyze. The reported Koc value for the third soil cannot be considered correct.

References

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9.0 BIODEGRADATION

Type: Aerobic [X]; Anaerobic [ ]
Inoculum: Adapted [ ]; Non-adapted [X]
Concentration of the chemical: 100 mg/L
Related to COD [ ]; DOC [ ]; Test substance [X];
Medium: Water [X]; Water-sediment [ ]; Soil [ ];
Sewage treatment [ ]
Contact time: 23 days at 24 $\pm$ 1°C
Degradation: 77%
Results: Readily biodeg. [X]; Inherently biodeg. [ ];
under test condition no biodegradation observed [ ], Other [ ]
Kinetic of test substance: (e.g. ZahN-Wellens-Test)

Day	Degradation of PI	Degradation of Positive Control
10	5%	40%
12	10% (Lag Phase)	58%
22	76% (10-day window)	78%
23	77%	78%

Control substance: Biotic:
Glucose (CAS RN 50-99-7) + Glutamic Acid (CAS RN 56-86-0)
Abiotic:
Mercuric chloride (CAS RN 7487-94-7)
Kinetic of control substance: See Above
Degradation Products: Yes [ ] No [ ] Not measured [X]
Method (Year): OECD Test Guideline 301F (1993)
GLP: Yes [X] No [ ] ? [ ]
Test Substance: N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-16 from General Electric Company; Purity: 99.8%

Remarks: Following is a summary of test conditions

Parameter	Test Condition
Test type	301F – Manometric Respirometer Test
Duration	23 days
Inoculum	Activated Sludge
Temperature	24 ± 1°C
COD Determination Method	Chemical oxidation with Hot Acidic Dichromate
BOD Determination Method	Manometric Respirometry
PI Determination Method	HPLC (Diode Array Detector)
Test vessel	2 L flasks
Test volume	1.5 L
Replicates	Two
Aeration	Oxygen
Controls	1. Test control (inoculum blank) 2. Procedure control (Reference substance plus inoculum) 3. Toxicity control (Reference substance, PI and inoculum)
Nominal PI concentration	100 mg/L
Criterion for Ready Biodegradability	60% COD/BOD in 10-d window within 28 days

Measured and Expected Concentrations of N-Methylphthalimide (PI) in Definitive Test Solutions

Flask	Flask Label	Nominal PI (mg/L)x	Measured PI at Test Initiation (mg/L) x_i	Relative Error (%) E_r	Measured PI at Test Termination (mg/L)
1	Test Solution #1	100	57.3	-42.7	<1.0
2	Test Solution #2	100	55.5	-44.5	<1.0
3	Inoculum Blank #1	0	<1.0	0	<1.0
4	Inoculum Blank #2	0	<1.0	0	<1.0
5	Reference Solution	0	<1.0	0	<1.0
6	Toxicity Control	100	61.5	-38.5	<1.0
7	Abiotic Control	100	61.3	-38.7	6.2

Chemical Oxygen Demand (COD) for Flasks at Test Initiation and Termination

Flask #	Flask Label	Initiation (mg O_2 / L)	Termination (mg O_2 / L)
1	Test Solution #1	164	<10
2	Test Solution #2	171	<10
3	Inoculum Blank #1	<10	<10
4	Inoculum Blank #2	<10	<10
5	Reference Solution	90	<10
6	Toxicity Control	256	21
7	Abiotic Control	180	212

5-d Biochemical Oxygen Demand (BOD_5) for Flasks at Test Initiation and Termination

Flask #	Flask Label	Initiation (mg O_2 / L)	Termination (mg O_2 / L)
1	Test Solution #1	<10	<10
2	Test Solution #2	<25	<10
3	Inoculum Blank #1	<10	<10
4	Inoculum Blank #2	<25	<10
5	Reference Solution	18	<10
6	Toxicity Control	18	<10
7	Abiotic Control	<25	<10

Reference: Eickhoff, C. (2002). Unpublished report no. 2-11-0868 entitled "Manometric Respirometry Test of N-Methylphthalimide [CAS RN 550-44-7]: Using OECD Guideline 301F)", dated February 19, 2002 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

ECOTOXICOLOGICAL DATA

10.0 ACUTE/PROLONGED TOXICITY TO FISH

10.0.1

Type of Test: Static [X] Semi-static [ ] Flow-through [ ] Other [ ]
Open-system [ ] Closed-system [ ]

Species: Rainbow trout (*Oncorhynchus mykiss)* (Spring Valley Trout Hatchery, Langley, British Columbia)

Exposure Period: 96 Hours

Results: LC_{50} (96h) 110 mg/L (95% CI = 93 – 131 mg/L)
LC_{50} (72h) 110 mg/L (95% CI = 93 – 131 mg/L)
LC_{50} (48h) 110 mg/L (95% CI = 93 – 131 mg/L)
LC_{50} (24h) 125 mg/L (95% CI = 111 – 140 mg/L)
NOEC = 58.7 mg/L (predicted based on linear regression analysis of nominal and measured concentrations)
LOEC = 100.3 mg/L (measured)

Analytical Monitoring: Yes [X] No [ ] ? [ ]

Method: OECD Test Guideline 203 (1993)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-16 from General Electric Company; Purity: 99.8%

Remarks: Test conditions: The fish were held 31 or 36 days before initiating the test on PI. The dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated). The dilution water had a hardness of 18-19 mg $CaCO_3$/L, alkalinity of 17 mg $CaCO_3$/L, pH of 7.5, and a conductance of 43-46 µs/cm. For test solutions, a 400-mg/L stock solution was prepared by adding 9.6 g of PI to a total volume of 24 L of fish house control/dilution water in eight 3-L aliquots. Each 3-L aliquot of the stock solution was stirred overnight at 27°C. The solutions were cooled to 15°C prior to test initiation, using an ice-water bath. The eight aliquots of stock solution were thoroughly mixed together in a clean, 20-gal glass aquarium before preparing the test solutions. The pH of this stock solution, pH 6.5, was not adjusted.

Based on the results of a range-finding study, nominal concentrations of 0, 40, 68, 116, 200, and 293.3 mg/L were used for the definitive test. A negative control was also employed. A reference toxicant, phenol, was used to assess the relative sensitivity of the fish and the precision of data produced by the laboratory. Nominal concentrations of 5, 7, 10, 13, and 22 mg phenol/L were used. Measured PI concentrations of 34.6, 100.3, and 293.3 mg/L were determined by HPLC for the low, mid, and high concentrations and "measured" concentrations of 58.7 and 174.6 mg/L were extrapolated from the measured values prior to the exposures. These measured values were used to calculate the test endpoints. At test initiation, dissolved oxygen, temperature, and pH ranged from 8.7 to 9.9 mg/L, 14.4 to 14.8 ºC , and 6.6 to 7.5 units, respectively. At test termination, the temperature and pH of the test solutions were 14.7 –14.8ºC and 7.2 – 7.4, respectively. Dissolved oxygen levels ranged from 9.0 – 9.2 mg/L.

The following is a summary of test conditions:

Parameter	Test Condition
Test type	Static
Duration	96 hours
Test organism / size (weight / age)	Rainbow trout (*Oncorhynchus mykiss*) 4.6 ± 0.3 cm in length and 0.93 ± 0.21 g
Photoperiod	8-h dark and 16-h light
Light intensity	100 - 500 lux at the water surface
Temperature	15 ± 1 ºC
Dissolved oxygen	98% to100% air-saturation
Feeding	None
Test vessel	22-L pails fitted with polyethylene liners
Test volume	18 L
Loading density	9 fish per test vessel (0.7 g fish per liter)
Replicates	One
Aeration	Aerated to maintain at least 60% dissolved O_2
Controls	Dilution water
Nominal concentrations	0, 40, 68,116, 200, 293.3 mg/L
Measured concentrations	0, 34.6, 58.7, 100.3, 174.6, 293.3
Criterion for effect	Death
Calculated toxicity values	LC_{50} at 24, 48, 72, and 96 h

Results:

At the initiation of the test, fish in the 100.3, 174.6, and 293.3 mg/L test solutions exhibited signs of stress, including rapid gill movement, flaring of gills, loss of equilibrium, and violent twitching; these fish were quiescent after 5 min of exposure. Fish exposed to 34.6 and 58.7 mg/L appeared normal. After 60 min of exposure, all fish in the 34.6 and 58.7 mg/L appeared normal; fish in 100.3 and 174.6 mg/L were quiescent and fish in 293.3 mg/L were all dead. After 3 hours of exposure, the fish in 100.3 mg/L, were quiescent but still had some gill and fin movements; the fish in 174.6 mg/L were quiescent but had very little gill and fin movements. The fish exposed to the other concentrations appeared normal. The fish were observed after 6 hours of exposure. In the lowest test concentration, 34.6 mg/L, some fish appeared normal, while others had swollen abdominal regions. The fish exposed to 58.7 mg/L, had darkened pigmentation, rapid gill

movements, and were upright on the bottom of the aquarium. One fish in this concentration had loss of equilibrium and some fish had slightly swollen abdominal regions. The fish exposed to 100.3 mg/L were quiescent, but gill and fin movements were still visible. One fish had loss of equilibrium and was swimming perpendicular to the aquarium bottom. All fish also had darkened pigmentation and rapid gill movements at this test concentration. In 174.6 mg/L, 7 fish were dead and the two surviving fish were quiescent and had swollen abdominal regions and very little gill and fin movements. Elongated, white strings of feces were present in all test aquaria, except the control and 174.6 mg/L. After a 16-hour exposure, one fish in 100.3 mg/L and the two remaining fish in 174.6 mg/L were found dead. At 24 hours, the fish in 34.6 mg/L appeared normal; one fish was quiescent in 58.7 mg/L. In 100.3 mg/L, eight fish were quiescent. At 48 hours of exposure, a total of three fish were dead, one fish had a swollen abdominal region and six fish were quiescent in the 100.3 mg/L. All other fish appeared normal. After 72 hours of exposure, a total of three fish were dead, two quiescent fish and five fish had swollen abdominal regions in the 100.3 mg/L. All other fish appeared normal in the other test solutions. At test termination (96-hour exposure), all fish appeared normal in the control and at 34.6 to 58.7 mg/L. In the 100.3 mg/L test solution, the surviving fish had rapid gill movements and two fish were quiescent.

Rainbow Trout Mortalities (%) After a 96-h Exposure to N-Methylphthalimide (PI)

N-Methylphthalimide (PI) Concentration (mg/L)	Mortality (%)
Control	0
34.6	0
58.7	0
100.3	33.3
174.6	100
293.3	100

Reference: Eickhoff, C. (2002). Unpublished report no. 2-11-0868 entitled "Fish (Oncorhynchus mykiss) Acute Toxicity Test of Test of N-Methylphthalimide [CAS RN 550-44-7]: Using OECD Guideline 203)", dated February 19, 2002 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

11.0 TOXICITY TO AQUATIC PLANTS (E.G. ALGAE)

11.0.1

Species: Green algae (*Selenastrum capricornutum*); Department of Botany Culture Collection, University of Toronto.

End-point: Biomass [ ] Growth rate [X] Other [ ]

Exposure Period: 72 hours

Results: Growth:

EC_{50} (72h) = 57.0 mg/L (95% CI = 51.0 – 62.9 mg/L)

NOEC < 27.6 mg/L

LOEC = 27.6 mg/L

Analytical Monitoring: Yes [X] No [ ] ? [ ]

Method: OECD Test Guideline 201 (1993)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-16 from General Electric Company; Purity: 99.8%

Remarks: Test conditions: Based on the results of a range-finding study, nominal concentrations of 0, 46, 70, 110, 170, 260 and 400 mg/L were used for the definitive test. A reference toxicant, zinc sulfate, was used to assess the relative sensitivity of the algae and the precision of data produced by the laboratory. Nominal concentrations of 0.0001, 0.001, 0.01, 0.05, 0.1, 0.5 and 1 mg $ZnSO_4$/L were used. Measured PI concentrations of 27.6, 80.4, and 360.2 mg/L were determined for the nominal 46, 110 and 400 mg/L groups, respectively. "Measured" values of 46.8, 141.5, and 226.8 mg/L were extrapolated from the measured values prior to the exposures for the 70, 170 and 260 mg/L groups, respectively. The measured values were used to calculate the test endpoints. Growth inhibition was assessed as the decrease in cell numbers relative to controls. At 24, 48 and 72 hours, two cell counts were performed on samples from each flask using a haemocytometer. The following is a summary of the test conditions:

Parameter	Conditions
Test species	*Selenastrum capricornutum*); Department of Botany Culture Collection, University of Toronto.
Duration of test	72 hours
Culture medium	*Selenastrum* nutrient media (SNM) prepared in deionized water
Testing medium	*Selenastrum* nutrient media (SNM) prepared in deionized water
Temperature	23 ± 2°C
Photoperiod	Continuous
Light intensity	6350 and 8180 lux
Test vessel	250 mL Erlenmeyer flasks
Nutrient/test solution volume	100 mL
pH of the test solutions	7.5
Age of test plants	6 days at test initiation
Number of cells per test vessel	1 x 10^6 (± 10%)
Range-finding test concentrations	0, 0.4, 40 and 400 mg/L
Definitive test concentrations	0, 46, 70, 110, 170, 260 and 400 mg/L
Measured concentrations	0, 26.9, 45.8*, 78.2*, 126.0, 199.9* and 313.7 mg/L
Number of replicate test vessels/concentration in definitive test	3 replicates per test concentration 6 replicates for control
Measured water quality parameters	pH at start and end of the test
Measured endpoints	Cell counts measured daily
Calculated endpoints	Area under the growth curve, growth rate, cell number
Test validity criteria	The number of algal cells in the controls increased by a factor of more than 16 in 72 hours.

* Predicted values based on linear regression analysis of nominal and measured concentrations.

Results: The pH of the test solutions were within the guideline specified range of 6.0 to 8.5 throughout the study with decreases of 1.5 to 1.8 pH units occurring during the study. The initial and final control cell densities were 9900 cells/mL and 658,000 cells/mL, respectively. This was a 66-fold increase in cell density over the 72-hour test period. A 16-fold increase was required for a valid test. The criterion for effect was growth inhibition based on a decrease in the area under the growth curves for each concentration relative to controls. The results of the cell counts and area under the growth curve are summarized in the table below. The EC_{50} for the reference toxicant, $ZnSO_4$, was 0.43 and 0.88 in two tests.

Summary of Cell Counts, Area Under Growth Curves and Cell Growth for *Selenastrum* Definitive Test

Measured Concentration (mg/L)	Average Cell Counts (x 10^4 / mL) 0 h	24 h	48 h	72 h	Mean Area Under Growth Curve	Mean Relative Cell Growth (%)
0	0.99	3.7	17.7	65.8	1242	100
27.6	0.99	2.5	14.2	58.7	1045	84
46.8	0.99	2.5	14.2	49.5	935	75
80.4	0.99	0.8	4.0	2.5	87	7
141.5	0.99	1.3	0.8	1.8	15	1
226.8	0.99	0.5	1.5	1.2	3	0
360.2	0.99	0.8	0.8	0.3	-15	-1

Reference: Eickhoff, C. (2002). Unpublished report no. 2-11-0868 entitled "Freshwater Alga, Selenastrum capricornutum, Growth Inhibition Test of N-Methylphthalimide [CAS RN 550-44-7]: Using OECD Guideline 201)", dated March 13, 2002 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

12.0 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

12.1.1 Daphnia

Type of Test: Static [X] Semi-static [ ] Flow-through [ ] Other [ ]
Open-system [ ] Closed-system [ ]

Species: *Daphnia magna*

Exposure Period: 24 Hours

Results: EC_{50} (24h) = 112 mg/L (95% CI = 102 – 122 mg/L)
NOAEC = 78.2 mg/L

Analytical Monitoring: Yes [X] No [ ] ? [ ]

Method: OECD Test Guideline 202 (1993)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: N-Methylphthalimide (PI, CAS RN 550-44-7); Lot PI-01-16 from General Electric Company; Purity: 99.8%

Remarks: Test conditions: The test was initiated with young daphnids less than 24 hours old. The culture vessels were incubated in a temperature-controlled room at 20 ± 1ºC, under an 8-hour dark and 16-hour light photoperiod. The light intensity at the water surface was 400 to 800 lux. Stock cultures were fed a mixture of a green alga (*Selenastrum capricornutum* and *Chlorella pyrenoidosa*) and a

solution of yeast, Cerophyll, and fermented trout chow three times weekly. Brood cultures were placed into new beakers with fresh culture water twice per week. At the time of toxicity testing, the *Daphnia* culture met the following health criteria: Ephippia were not present; age of delivery of first brood was less than 12 days; females 2-5 weeks old delivered and averaged of 15 or more neonates per brood; no more than 25% of the brood stock died during the 7-d period prior to the test, assuming a culture of mixed ages; and the results of the test with a reference toxicant or positive control (zinc sulphate) were acceptable, indicating that the organisms were responding normally to the reference compound. The dilution water had a hardness of 94 mg $CaCO_3$/L and was within 20% of the culture water (80 - 100 mg $CaCO_3$/L), alkalinity of 64 mg $CaCO_3$/L, and pH of 7.9. The ratios of calcium-to-magnesium and sodium-to-potassium on a weight-to-weight basis were 2:1 and 11:1 respectively. The oxygen content was within the range 90 to 100% air saturation. The control was laboratory dilution water. Based on the results of a range-finding study, nominal concentrations of 0, 46, 70, 110, 170, 260 and 400 mg/L were used for the definitive test. A reference toxicant, zinc sulfate, was used to assess the relative sensitivity of the algae and the precision of data produced by the laboratory. Nominal concentrations of 0.1, 0.32, 1.0, 3.2, 10, and 32 mg $ZnSO_4$/L were used. Measured PI concentrations of 26.9, 126.0 and 313.7 mg/L were determined for the nominal 46, 126 and 400 mg/L groups. "Measured" values of 45.8, 78.2, and 199.9 mg/L were extrapolated from the measured values prior to the exposures for the 46, 126 and 313.7 mg/L groups. The nominal values were used to calculate the test endpoints.

The following is a summary of test conditions:

Parameter	Test Condition
Test type	Static
Duration of test	24 hours
Test organism	*Daphnia magna*; Pacific Environmental Science Centre, N. Vancouver, BC.
Temperature	20 $\pm$ 1 ºC
Photoperiod	8-h dark and 16-h light
Light intensity	400 - 800 lux at the water surface
Dissolved oxygen	90% to100% air-saturation
Feeding	None
Test vessel	250 mL beakers
Test volume	200 mL
Replicates	4
Aeration	None
Controls	Dilution water
Nominal concentrations	0, 46, 70, 110, 170, 260 and 400 mg/L
Measured concentrations	0, 26.9, 45.8*, 78.2*, 126.0, 199.9* and 313.7 mg/L
Criterion for effect	Immobilization
Calculated toxicity values	EC_{50} at 24h

* Predicted values based on linear regression analysis of nominal and measured concentrations.

Borosilicate glass beakers (250-mL) were used for the test. Each contained 200 mL of test solution, which provided a loading density of greater than 2 mL of test solution for each test organism. Test vessels were covered during the test. Measurements of dissolved oxygen, temperature, and pH were made in each test solution, including control(s), at test initiation and termination after biological observations were completed. Initial measurements of conductivity and hardness were also measured in each test concentration and the control. Initial measurements were taken from a separate 'measurement beaker' to avoid contamination of the test solutions. This beaker was filled with the same solutions as the test beakers. Final measurements were taken in each replicate. The pH, hardness, alkalinity, and Ca/Mg and Na/K ratios of the *Daphnia* control/dilution water were also determined. General observations on behavior were made at test initiation, and at 30 minutes and 24 hours of exposure. Immobilization was defined as the inability to swim during 15 seconds following gentle agitation of the solution, even if the *Daphnia* could still move their antennae. Observations of daphnids were aided by a black background, by illumination from the side, and by the use of a microscope.

Results: At test initiation, dissolved oxygen, temperature, pH, and conductivity ranged from 8.3 to 9.1 mg/L, 19.5 to 20.7 ºC, 6.7 to 7.9 units, 342 to 348 µS/cm, respectively. At test termination, dissolved oxygen, temperature, and pH ranged from 8.3 to 9.1 mg/L, 19.5 to 20.7 ºC , and 7.0 to 7.7 units, respectively. After 24 hours, all *Daphnia* appeared normal at 0, 26.9, 45.8, and 78.2 mg/L. All *Daphnia* were alive with 75% immobilized and 25% able to swim at 126.0 mg/L. At 199.9 mg/L 50% of the *Daphnia* were dead and 50% were immobilized, and at 313.7 mg/L all *Daphnia* were dead. The following table summarizes the results:

Average Number (and Standard Deviation) of *Daphnia* Immobilised After a 24-hour Exposure to N-methylphthalimide (PI)

Nominal PI Concentration (mg/L)	Measured or Predicted PI Concentration (mg/L)	Average Immobilization (%)	Standard Deviation (%)
Control	Control	0	0
46	26.9	0	0
70	45.8	0	0
110	78.2	0	0
170	126.0	75	10
260	199.9	100	0
400	313.7	100	0

Reference: Eickhoff, C. (2002). Unpublished report no. 2-11-0868 entitled "Daphnia Magna Acute Immobilisation Test of N-Methylphthalimide [CAS RN 550-44-7]: Using OECD Guideline 202)", dated September 19, 2001 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

TOXICITY

13.0 ACUTE TOXICITY

13.1 ACUTE ORAL TOXICITY

Type:	LD_0 [ ] LD_{100} [ ] LD_{50} [X] LD_{L0} [ ] Other [ ]
Species/Strain:	Rat/Spartan
Value:	LD_{50} > 500 mg/kg
Method:	Method was not defined.
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); identified as "AR No. 83637" from General Electric Company, Schenetady, NY, USA; Purity: not provided
Remarks:	Two male albino rats of the Spartan strain were used at each of two dosage levels. The rats weighed from 201 to 210 grams at the initiation of the study period. The rats had food and water available *ad libitum* except for an overnight period preceding compound administration during which food, but not water, was withheld. The test material was administered to two rats each at dosage levels of 500 and 5000 mg/kg. The test compound was suspended in corn oil at concentrations enabling the administration of 10 mL/kg at both dosage levels. All rats were observed for mortality for a period of 14 days. Body weights were measured initially and at 14 days. Results: Neither of the rats at the 500 mg/kg dosage level died during the 14-day postdosing observation period. Both rats exhibited normal body weight gains. One rat that received 5000 mg/kg of the test substance died during the 14-day postdosing period. Details on the time of death were not available. The surviving rat at this dose level exhibited a lesser body weight gain than did the survivors at the 500 mg/kg dosage level. Based on the results, PI would be considered a possible toxic material by the oral route of administration.
Reference:	Wazeter, F.X. and E. I. Goldenthal (1974) Unpublished report no. 313-047 entitled "Acute Toxicity Screening Studies in Rats and Rabbits", dated October 2, 1974 for General Electric Company; from International Research and Development Corporation, Mattawan, MI, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Minimum number of animals tested. A definitive LD_{50} value could not be calculated but the data provide information on the acute oral toxicity of PI.

13.2 ACUTE INHALATION TOXICITY

No studies were found.

13.3 ACUTE DERMAL TOXICITY

Type:	LD_0 [ ] LD_{100} [ ] LD_{50} [X] LD_{L0} [ ] Other [ ]
Species/Strain:	Rabbit/New Zealand White
Value:	LD_{50} > 2000 mg/kg
Method:	Method was not defined.
GLP:	Yes [X] No [ ] ? [ ]

Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); identified as "AR No. 83637" from General Electric Company, Schenetady, NY, USA; Purity: not provided
Remarks:	Two New Zealand White rabbits (one male and one female) were used at each of two dosage levels. The rabbits weighed from 2760 to 2952 grams at the start of the study period. Food and water were available *ad libitum*. Body weights were measured initially and at 14 days after compound application. The hair was removed from the back of each rabbit with an electric clipper. The test substance was applied once only to the back of each rabbit. Two rabbits received 200 mg/kg and two rabbits received 2000 mg/kg of the test substance. The area of application was then wrapped with a gauze bandage and occluded with Saran Wrap. After a 24-hour exposure period, the bandages were removed and the backs were washed with tepid tap water. The rabbits were observed for mortality for a period of 14 days. Results: Neither of the rabbits at either dose level died during the 14-day post-exposure observation period. All four of the rabbits exhibited body weight gains during this time period. Based upon the results obtained, PI would not be considered a toxic material by the dermal route of administration.
Reference:	Wazeter, F.X. and E. I. Goldenthal (1974) Unpublished report no. 313-047 entitled "Acute Toxicity Screening Studies in Rats and Rabbits", dated October 2, 1974 for General Electric Company; from International Research and Development Corporation, Mattawan, MI, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Minimum number of animals tested. A definitive LD_{50} value could not be calculated but the data provide information on the acute dermal toxicity of PI.

14.0 GENETIC TOXICITY *IN VITRO* OR *IN VIVO* (CHROMOSOMAL ABERRATIONS)

14.1

Type:	*In vitro* mammalian chromosome aberration test
System of testing:	Chinese hamster ovary (CHO) cells
Concentration:	0, 402.5, 805, 1610 µg/mL (4-hr treatment w/o S9 mix) 0, 402.5, 805, 1610 µg/mL (20-hr treatment w/o S9 mix) 0, 402.5, 805, 1610 µg/mL (4-hr treatment with S9 mix)
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Negative; with and without activation
Cytotoxicity conc:	With metabolic activation: > 1610 µg/mL Without metabolic activation: > 1610 µg/mL
Precipitation conc:	With metabolic activation: > 1610 µg/mL Without metabolic activation: > 1610 µg/mL
Genotoxic effects:	+ ? - With metabolic activation: [ ] [ ] [X] Without metabolic activation: [ ] [ ] [X]
Method:	OECD Test Guideline 473 (1998)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7; Lot #PI-01-16 from General Electric Company); Purity: 99.8% .
Remarks:	Description of test procedure: A preliminary toxicity assay was performed for the purpose of selecting doses for the chromosome

aberration assay and consisted of an evaluation of test article effect on cell growth. CHO cells were seeded for each treatment condition at approximately 5 x 10^5 cells/25 cm^2 flask and were incubated at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air for 16-24 hours. Treatment was carried out by refeeding the flasks with 5 mL complete medium (McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or S9 reaction mixture (4 mL serum-free medium plus 1 mL of S9/cofactor pool) for the activated study, to which was added 50 µL dosing solution of test article in solvent or solvent alone. The osmolality of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. The cells were treated for 4 hours with and without S9, and continuously for 20 hours without S9. At completion of the 4-hour exposure period, the treatment medium was removed, the cells washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS), refed with 5 mL complete medium and returned to the incubator for a total of 20 hours from the initiation of treatment. At 20 hours after the initiation of treatment the cells were harvested by trypsinization and counted using a Coulter counter. The presence of test article precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control. In the preliminary toxicity assay, the maximum dose tested was 1610 µg/mL. The test article was soluble in treatment medium at all doses tested. Selection of doses for the chromosome aberration assay was based on cell growth inhibition relative to the solvent control. No substantial toxicity, i.e., at least 50% cell growth inhibition, was observed at any dose in the non-activated 4- and 20-hour exposure groups or in the S9-activated 4-hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 201.25 to 1610 µg/mL for the non-activated 4- and 20-hour exposure groups and for the S9-activated 4-hour exposure group.

For the chromosome aberration assay, CHO cells were seeded at approximately 5 x 10^5 cells/25 cm^2 flask and were incubated at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air for 16 to 24 hours. Treatment was carried out by refeeding duplicate flasks with 5 mL complete medium (McCoy's 5A medium supplemented with 10% FBS, 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or 5 mL S9 reaction mixture for the S9 activated study, to which was added 50 µL of dosing solution of test or control article in solvent or solvent alone. The osmolality of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. In the non-activated study, the cells were exposed to the test article continuously for 4 or 20 hours After the exposure period for the 4-hour exposure group, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and

returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks returned to the incubator until cell collection. In the S9 activated study, the cells were exposed for 4 hours. After the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks were returned to the incubator until cell collection. Two hours after the addition of Colcemid®, metaphase cells were harvested for both the non-activated and S9 activated studies by trypsinization. Cells were collected approximately 20 hours after initiation of treatment. The cells were collected by centrifugation at approximately 800 rpm for 5 minutes. The cell pellet was resuspended in 2-4 mL 0.075 M potassium chloride (KCl) and allowed to stand at room temperature for 4-8 minutes. The cells were collected by centrifugation, the supernatant aspirated and the cells fixed with two washes of approximately 2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v). The cells were stored overnight or longer in fixative at approximately 2-8°C. To prepare slides, the fixed cells were centrifuged at approximately 800 rpm for 5 minutes, the supernatant was aspirated, and 1 mL fresh fixative was added. After additional centrifugation (at approximately 800 rpm for 5 minutes) the supernatant fluid was decanted and the cells resuspended to opalescence in fresh fixative. A sufficient amount of cell suspension was dropped onto the center of a glass slide and allowed to air dry. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

Evaluation of metaphase cells: Slides were coded using random numbers by an individual not involved with the scoring process. To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Metaphase cells with 20 ± 2 centromeres were examined under oil immersion without prior knowledge of treatment groups. Initially, the non-activated and S9-activated 4-hour exposure groups were evaluated for chromosome aberrations and since a negative result was obtained in the non-activated 4-hour exposure group, the non-activated 20-hour continuous exposure group was then also evaluated for chromosome aberrations. A minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) were also recorded. Chromatid and isochromatid gaps

were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using a calibrated microscope stage. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored.

Evaluation of test results: The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell were calculated and reported for each group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness. All conclusions were based on sound scientific basis; however, as a guide to interpretation of the data, the test article is considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

Criteria for evaluating results: The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

Plates/test: Samples were run in duplicate, with and without metabolic activation.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Negative and Positive controls: Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 μg/mL. Cyclophosphamide (CP) was used as the positive control in the S9 activated study at final concentrations of 10 and 20 μg/mL. The solvent vehicle (acetone) for the test article was used as the solvent control at the same concentration as that found in the test article-treated groups.

Results: In the chromosome aberration assay, the test article was soluble in treatment medium at all doses tested. The osmolality in the treatment medium of the highest concentration tested (1610 μg/mL), was 299 mmol/kg. The osmolality of the solvent (acetone) in treatment medium was 304 mmol/kg. The pH of the highest concentration of test article in treatment medium was approximately 7.5.

4-hour harvest without metabolic activation: Toxicity of N-Methylphthalimide in CHO cells when treated for 4 hours in the absence of S9 activation was 11% at 1610 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest dose evaluated for chromosome aberrations, 1610 µg/mL, was reduced 4% relative to the solvent control. The doses selected for microscopic analysis were 402.5, 805, and 1610 µg/mL. The percentage of cells with structural and numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control, regardless of dose ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (14.5%) was found to be statistically significant.

4-hour harvest with metabolic activation: Toxicity of N-Methylphthalimide in CHO cells when treated for 4 hours in the presence of S9 activation was 14% at 1610 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest dose evaluated for chromosome aberrations, 1610 µg/mL, was reduced 10% relative to the solvent control. The doses selected for microscopic analysis were 402.5, 805, and 1610 µg/mL. The percentage of cells with structural aberrations in the test article-treated groups was significantly increased above that of the solvent control at 1610 µg/mL ($p\leq0.05$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p<0.05$). However, the percent aberrant cells in the test article-treated group (6.5%) was within the historic solvent control range of 0.0% to 6.5%. Therefore, the increase in percentage of cells with structural aberrations is not considered to be biologically significant. The percentage of cells with numerical aberrations in the test article-treated groups was not statistically increased above that of the solvent control, regardless of dose ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) treatment group (29.5%) was found to be statistically significant.

20-hour harvest without metabolic activation: In the absence of a positive response in the non-activated 4-hour exposure group, slides from the non-activated 20-hour exposure group were evaluated for chromosome aberrations. Toxicity of N-Methylphthalimide was 1% at 1610 µg/mL, the highest test concentration evaluated for chromosome aberrations in the non-activated 20-hour continuous exposure group. The mitotic index at the highest dose evaluated for chromosome aberrations, 1610 µg/mL, was reduced 35% relative to the solvent control. The doses selected for microscopic analysis were 402.5, 805, and 1610 µg/mL. The percentage of cells with structural and numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control, regardless of dose ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (16.5%) was found to be statistically significant.

Overall Conclusion: The positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay, PI was concluded to be negative for the induction of structural and numerical chromosome aberrations in Chinese hamster ovary cells.

Summary of Test Results

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean ± SD)	Cells with Numerical Aberrations (%)	Cells with Structural Aberrations (%)
Vehicle (Acetone)	-	4	11.3	200	0.005 ± 0.071	2.5	0.5
N-Methylphthalimide							
402.5	-	4	11.2	200	0.010 ± 0.141	2.5	0.5
805	-	4	11.0	200	0.005 ± 0.071	3.5	0.5
1610	-	4	10.9	200	0.015 ± 0.158	4.0	1.0
Positive control (MMC) 0.2	-	4	12.4	200	0.170 ± 0.471	3.5	14.5**
Vehicle (Acetone)	+	4	12.2	200	0.020 ± 0.140	3.5	2.0
N-Methylphthalimide							
402.5	+	4	11.9	200	0.040 ± 0.221	3.5	3.5
805	+	4	11.5	200	0.040 ± 0.196	3.5	4.0
1610	+	4	11.0	200	0.075 ± 0.300	4.5	6.5*
Positive control (CP) 10	+	4	7.2	200	0.420 ± 0.766	2.5	29.5**
Vehicle (Acetone)	-	20	11.3	200	0.005 ± 0.071	1.5	0.5
N-Methylphthalimide							
402.5	-	20	9.5	200	0.005 ± 0.071	2.5	0.5
805	-	20	10.8	200	0.010 ± 0.100	3.5	1.0
1610	-	20	7.4	200	0.020 ± 0.140	3.0	2.0
Positive control (MMC) 0.1	-	20	9.3	200	0.210 ± 0.507	4.5	16.5**

Treatment: Cells from both the 4-hour and 20-hour treatment regimens were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: * $p \leq 0.05$; ** $p \leq 0.01$; using the Fisher's exact test.

Reference: Gudi, R., C. Brown. (2001). Unpublished report no AA44TV.331.BTL entitled "In vitro mammalian chromosome aberration test" dated October 4, 2001 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.
Reliability: (Klimisch Code 1) Valid without restrictions.

15.0 GENETIC TOXICITY *IN VITRO*

15.1 BACTERIAL TEST

15.1.1
Type: Bacterial reverse mutation assay (Ames test)
System of testing: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538
Concentration: 0, 1, 10, 100, 500, 1000, 2500, 5000 and 10000 µg/plate
Metabolic activation: With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results: Negative
Cytotoxicity conc.: Slightly toxic to TA1537 at 10,000 µg per plate.

Precipitation conc.:	Not mentioned
Genotoxic effects:	+ ? -
	With metabolic activation: [ ] [ ] [X]
	Without metabolic activation: [ ] [ ] [X]
Method:	Ames, B.N. et al (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res., 31:347-364.
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7; identified as 02-81-011535-0`0 AR #92477 from General Electric Company); Purity: not provided.
Remarks:	Description of test procedure: The test system was exposed to the test substance via the plate incorporation methodology originally described by Ames et al. (1975). The nonactivation assay was performed by placing a sterile 13 x 100 mm test tube in a 43°C water bath and adding the following items in order: 1) 2.00 mL of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin; 2) 0.05 mL of a solution of the test substance to give the appropriate dose; 3) 0.1 mL to 0.2 mL of indicator organism; 4) 0.5 mL of 0.2M phosphate buffer, pH 7.4. The mixture was swirled gently and then poured onto minimal agar plates. After the top agar had set, the plates were incubated at 37°C for approximately 2 days. The number of his+ revertant colonies growing on the plates were counted. For the activation assay, the only difference is the addition of 0.5 mL of S9 mix to the tubes in place of 0.5 mL of phosphate buffer which was added in the nonactivation assays. All other details are similar to the procedure for the nonactivation assays. *Criteria for evaluating results*: The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. In addition, for strains TA1535, TA1537 and TA1538, if the solvent control value is within the normal range, a test substance producing a positive response equal to three times the solvent control value is considered mutagenic. For strains TA98 and TA100, if the solvent control value is within the normal range, a test substance producing a positive response equal to twice the solvent control value is considered mutagenic. *Plates/test*: Duplicate plates were run for the TA1535 tester strain while all other species were run with one plate. *Activation system:* Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. *Negative and Positive controls:* Dimethylsulfoxide (DMSO) was the solvent for the test substance and served as the negative control. For the non-activation assay, the following positive control substances were used: Sodium azide (for strains TA1535 and TA100); 2-Nitrofluorene (for strains TA1538 and TA98) and 9-aminoacridine (for strain TA1537). The positive control substance, 2-anthramine was used for all tester strains with metabolic activation. *Results*: The presence of precipitate was not mentioned in the report. Slight toxicity was observed in strain TA1537 at the highest concentration tested, 10000 µg/plate. In the mutagenicity assay, no positive response was observed with any of the tester strains in the

presence or absence of S9 activation. The results are summarized in the following tables:

Revertants Per Plate
Liver Microsomes: None

Dose (µg/plate)	TA1535[a]	TA1537	TA1538	TA98	TA100
Solvent (DMSO)	11	4	14	38	97
Solvent (DMSO)	12	3	17	32	91
1	12	9	12	25	87
10	11	8	11	36	118
100	13	8	11	23	114
500	9	8	10	21	122
1000	12	7	12	33	107
2500	11	7	16	22	110
5000	11	5	10	29	96
10000	8	1	16	39	68
Positive Control	769	103	973	891	1413
Positive Control	849	95	1096	944	1776

[a] Values for TA1535 represent an average of the scores from two plates.

Revertants Per Plate
Liver Microsomes: Rat Liver S9

Dose (µg/plate)	TA1535[a]	TA1537	TA1538	TA98	TA100
Solvent (DMSO)	10	5	15	38	113
Solvent (DMSO)	12	4	19	27	120
1	16	9	19	23	109
10	12	5	18	22	133
100	10	6	13	33	128
500	14	9	11	41	130
1000	11	4	14	28	127
2500	10	10	12	37	110
5000	10	9	17	34	104
10000	9	7	20	40	106
Positive Control	261	115	1216	991	1649
Positive Control	235	134	1165	1080	1600

[a] Values for TA1535 represent an average of the scores from two plates.

Conclusion: The test substance did not exhibit genetic activity in any of the assays conducted on this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D.R. and Brusick, D. J. (1981) Unpublished report for Project no. 20988 entitled "Mutagenicity evaluation of 02-81-011535-010 AR #93477 in the Ames Salmonella/Microsome Plate Test" dated April 1981 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc. Kensington, MD, USA.

Reliability: (Klimisch Code 2) Reliable with restrictions. The test was conducted with only one plate per dose level for strains TA1537, TA1538, TA98 and TA100. Current guidelines require duplicate or triplicate plating.

15.1.2

Type:	Bacterial reverse mutation assay (Ames test)
System of testing:	*Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 and *Saccharomyces cerevisiae* (strain D4)
Concentration:	0, 0.1, 1, 10, 100 and 500 µg/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Negative
Cytotoxicity conc.:	Toxic to TA1535, TA1537, TA1538 and TA98 at 500 µg per plate.
Precipitation conc.:	Not mentioned
Genotoxic effects:	

	+	?	-
With metabolic activation:	[ ]	[ ]	[X]
Without metabolic activation:	[ ]	[ ]	[X]

Method:	Ames, B.N. et al (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res., 31:347-364.
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7; identified as 02-81-011535-0`0 AR #92477 from General Electric Company); Purity: not provided.
Remarks:	Description of test procedure: The test system was exposed to the test substance via the plate incorporation methodology originally described by Ames et al. (1975). The nonactivation assay was performed by placing a sterile 13 x 100 mm test tube in a 43°C water bath and adding the following items in order: 1) 2.00 mL of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin; 2) 0.05 mL of a solution of the test substance to give the appropriate dose; 3) 0.1 mL to 0.2 mL of indicator organism; 4) 0.5 mL of 0.2M phosphate buffer, pH 7.4. The mixture was swirled gently and then poured onto minimal agar plates. After the top agar had set, the plates were incubated at 37°C for approximately 2 days. The number of his+ revertant colonies growing on the plates were counted. For the activation assay, the only difference is the addition of 0.5 mL of S9 mix to the tubes in place of 0.5 mL of phosphate buffer which was added in the nonactivation assays. All other details are similar to the procedure for the nonactivation assays. *Criteria for evaluating results*: The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. In addition, for strains TA1535, TA1537 and TA1538, if the solvent control value is within the normal range, a test substance producing a positive response equal to three times the solvent control value is considered mutagenic. For strains TA98 and TA100, if the solvent control value is within the normal range, a test substance producing a positive response equal to twice the solvent control value is considered mutagenic. *Plates/test*: Duplicate plates were run for the TA1535 tester strain while all other species were run with one plate. *Activation system:* Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. *Negative and Positive controls:* Dimethylsulfoxide (DMSO) was the solvent for the test substance and served as the negative control. For the non-activation assay, the following positive control substances

were used: Sodium azide (for strains TA1535 and TA100); 2-Nitrofluorene (for strains TA1538 and TA98) and 9-aminoacridine (for strain TA1537). The positive control substance, 2-anthramine was used for all tester strains with metabolic activation.
Results: The presence of precipitate was not mentioned in the report. Slight toxicity was observed in strain TA1537 at the highest concentration tested, 10000 µg/plate. In the mutagenicity assay, no positive response was observed with any of the tester strains in the presence or absence of S9 activation. The results are summarized in the following tables:

Revertants Per Plate
Liver Microsomes: None

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4
Solvent (DMSO)	10	18	13	28	169	32
0.1	18	17	19	24	181	62
1	10	12	13	33	208	45
10	9	20	14	34	214	44
100	12	16	15	28	207	45
500	10	12	16	32	220	44
Positive Control	653	564	>1000	>1000	>1000	573

Revertants Per Plate
Liver Microsomes: Rat Liver S9

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4
Solvent (DMSO)	17	13	24	35	267	23
0.1	10	11	16	44	289	22
1	11	10	11	39	243	19
10	13	14	18	33	248	23
100	5	9	21	35	259	30
500	9	11	12	26	287	17
Positive Control	131	213	574	891	831	48

Conclusion: The test substance did not exhibit genetic activity in any of the assays conducted on this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D.R. and Brusick, D. J. (1977) Unpublished report for Project no. 20838 entitled "Mutagenicity evaluation of 09-77-011154-016" dated October 1977 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc. Kensington, MD, USA.

Reliability: (Klimisch Code 2) Reliable with restrictions. The test was conducted with only one plate per dose level for strains TA1537, TA1538, TA98 and TA100. Current guidelines require duplicate or triplicate plating.

16.0 REPEATED DOSE TOXICITY

16.1

Species/Strain: Rat/Sprague-Dawley
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Oral, Dietary feed
Exposure Period: 30 days

Frequency of Treatment:	Daily
Post Exposure Observation Period:	None
Dose:	0, 0.25, 0.5 and 1.0% of PI in basal diet
Control Group:	Yes [X] No [ ] No data [ ] Concurrent no treatment [X] Concurrent vehicle [ ] Historical [ ]
NOAEL:	0.25%
LOAEL:	0.5%
Method:	Groups of 10 rats/sex were fed PI at levels of 0, 0.25, 0.5 and 1.0%. All rats were observed for mortality twice each day. Clinical signs and body weights were recorded at initiation and weekly thereafter. Food consumption was recorded weekly. After 30 days of treatment, all surviving rats were fasted overnight and weighed prior to terminal sacrifice and necropsy. At necropsy the liver and kidneys were weighed. The following tissues were saved from all animals: brain, pituitary, thoracic spinal cord, eyes, salivary glands (mandibular), stomach, trachea, thymus esophagus, heart, spleen, adrenals, pancreas, duodenum, jejunum, ileum, colon, cecum, mesenteric lymph node, urinary bladder, testes with epididymides and prostate (males), ovaries and uterus (females), femur, bone marrow (costochondral junction), lungs, liver, kidneys, thyroid (with parathyroids), skeletal muscle, and all gross lesions. Microscopic evaluation was conducted on sections of the lungs, liver, brain and kidneys from rats of all treatment groups. A necropsy also was performed on all rats that were sacrificed in extremis or were found dead during the study. Reproductive organs were not evaluated histologically. The following statistical tests were utilized to evaluate body weight changes, total food consumption and organ weights: Bartlett's test for homogeneity of variance and one-way classification analysis of variance. If the ANOVA was heterogeneous, a $\log_{10}$ transformation was performed, followed by Bartlett's test. If the $\log_{10}$ transformation was ineffective in removing variance heterogeneity, ANOVA of the untransformed data was completed. When the ANOVA indicated homogeneous data, the Scheffe multiple comparison procedure was used to compare the control and treatment group means. When the ANOVA indicated heterogeneous data, Games and Howell's modification of the Tukey-Kramer honestly significant difference test was used to compare the group means.
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); Lot UI-82-2 from General Electric Company; Purity: not specified
Results:	One male rat in the 0.25% group died on Day 26 of the study. Statistically significant decreases in body weight of both male and female rats in the 0.5 and 1.0% groups (6% and 18% lower than controls for males and 4% and 11% lower than control for females, respectively) were noted during the fourth week of exposure. Mean food consumption values of the treated male and female rats were lower than control at some measurement periods, but the food consumption value for the male rats in the 1.0% group during the final week of exposure was the only statistically significant decrease noted. Mean compound consumption for the male rats in the 0.25, 0.50 and 1.0% groups ranged from 146 to 203 mg/kg/day, 330 to 403 mg/kg/day and 685 to 815 mg/kg/day, respectively. The mean

	compound consumption for the female rats in the 0.25, 0.5 and 1.0% groups range from 197 to 226 mg/kg/day, 409 to 573 mg/kg/day and 921 to 1100 mg/kg/day, respectively. The gross pathology revealed no treatment-related effects in any PI-treated group. Mean liver relative to terminal body weight was increased for both sexes and mean kidney weight relative to terminal body weight was increased for males at 1.0%. Microscopic evaluation revealed compound-related findings of centrilobular to diffuse hepatocellular enlargement in rats of both sexes in the 0.5 and 1.0% treatment groups. In addition, the severity of chronic progressive nephropathy was increased slightly in the male rats in the 1.0% group. There were no compound-related findings present in the tissues examined from the male or female rats in the 0.25% group.
Reference:	Burdock, G.A. (1983). Unpublished report no 349-261 entitled "30-Day Subchronic Oral Toxicity Study in Rats, PI and 4-NPI" dated January 21, 1983 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Minimal data collected for a repeat dose study.

17.0 REPRODUCTIVE TOXICITY

No studies were found.

18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1

Species/Strain:	Rabbit, New Zealand White
Sex:	Female [X]; Male [ ]; Male/Female [ ]; No data [ ]
Route of Administration:	Oral (gavage)
Duration of Test:	29 days
Exposure Period:	Days 6 through 18 of gestation
Frequency of Treatment:	Daily
Dose:	500 mg/kg/day
Control group:	Yes [X] No [ ] No data [ ] Concurrent no treatment [ ] Concurrent vehicle [X] Historical [ ] Positive Control (thalidomide)
NOEL Maternal Toxicity:	Not established (based on decrease in body weight gain)
NOEL Teratogenicity:	> 500 mg/kg/day
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	N-Methylphthalimide (PI, CAS RN 550-44-7); Lot UI-82-2 from General Electric Company; Purity: not specified
Method:	Ninety mature New Zealand White female rabbits were obtained from Dutchland Laboratory Animals, Inc., Denver, PA for use in this study. The animals were acclimated for a minimum of 22 days prior to the initiation of the study. During the period of acclimation, the rabbits were examined for general health and appearance. The animals were uniquely identified by ear tag and provide commercial rabbit ration (Purina lab Rabbit Chow®) and tap water *ad libitum*. The environment of the study room was maintained at 70 – 78 ºC , relative humidity of 53-86% and a 12-hour light/dark cycle. At Day 0 of

gestation, the body weights ranged from 2845 to 4700 grams. The animals were artificially inseminated with sperm from the laboratory breeding stock five hours after induction of ovulation with chorionic gonadotropin.. Five groups were included in this study; for the purposes of this summary, only three groups (control, positive control, and PI treated) will be discussed. Sixteen animals per group (to obtain at least 12 pregnant) were treated with vehicle (0.5% carboxymethyl cellulose), positive control (thalidomide; 150 mg/kg/day) or PI (500 mg/kg/day). Thalidomide and PI were suspended in vehicle to provide a dose volume of 1.5 ml/kg and 1.25 ml/kg, respectively. Control dose volume was 4.0 ml/kg. The dose was administered from gestation day (gd) 6 through 18, approximately the same time each day, and was based on each individual body weight on gd 6 (starting on gd 11, three animals in the control group and four animals in each of the thalidomide and PI groups were dosed based on gd 11 body weight). All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights were recorded on gd 0, 6, 11, 15, 19, and 29. Individual food consumption was recorded weekly. On gd 29, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live, dead, and early and late resorbed fetuses; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, weighed and measured from the frontal-parietal suture to the base of the tail (crown-rump distance). Casarean sections were also performed on dams that were found dead, sacrificed moribund or sacrificed due to early delivery. The number of corpora lutea, implantations, resorptions and live or dead fetuses was recorded.

Visceral Examination of Fetuses: The unfixed fetuses underwent visceral examination according to the method of Staples. All of the fetuses were opened by longitudinal incision, the sex determined and examined grossly both externally and internally. Major organs were inspected *in situ* with special attention to the heart and major blood vessels. The heads of approximately one-third of the fetuses were removed, fixed in Bouin's solution, sectioned by Wilson's freehand sectioning technique for examination of the eyes, palate, nasal septum and brain. The prepared sections were then re-examined against a light box with the aid of magnification.

Skeletal Examination of Fetuses: Following visceral examination, all fetuses (minus the head for approximately one-third of the fetuses) were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies. Examinations were performed with the aid of magnification on a light box.

Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be

homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Games and Howell modification of the Tukey-Kramer honestly significant difference test was used to compare groups. Pregnancy rates were analyzed by Fisher's exact test. External, visceral, and skeletal anomalies were evaluated by a multiple proportions test. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights and lengths with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.
Range-finding study: A range-finding study was conducted to select the dose used in this study. Four New Zealand White rabbits were dosed with PI at 1000 mg/kg/day from days 1 through 6 and at 500 mg/kg/day from days 10 through 16 of the study. Two animals died, one on day 9 and the second on day 12. Based on this study, 500 mg/kg/day was selected for the teratology study.

Results: Weight loss was observed in the thalidomide- and PI-treated groups during the treatment period. Statistical evaluation of body weight change did not, however, reveal any significant differences between treated and control groups. No effects on food consumption or gross pathology of the dams were observed. The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	PI (500 mg/kg/day)
Number of dams	16	16	16
Number pregnant	14	16	15
Pregnancy rate (%)	88	100	94
# dams surviving to gd 29 (survival rate)	13 (93%)	15* (100%)	13 (87%)
Mean number of			
Corpora lutea	13.4	12.2	11.5
Implantations	9.4	8.3	7.7
Resorptions-total	1.2	5.3	0.8
Fetuses – live	7.5	3.4	6.7
– dead	0.5	0	0
Indices (mean per litter)			
Implantation efficiency (%)	73.6	68.1	67.7
Incidence of resorption (%)	17.2	61.0	18.7
Incidence of fetal mortality (%)	3.8	0	0
Incidence of fetal viability (%)	79.2	39.1	81.4
Live fetuses			
Mean body weight (g) – males	40.91	38.36	42.88
– females	39.90	37.60	41.01
Mean length (cm) – males	9.49	9.03	9.51
– females	9.33	8.92	9.33
Percent Males	51.5	58.0	46.1
Mean uterine weights – gravid (g)	485.3	228.3	395.5

* One animal died accidentally on gd 8

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	PI (500 mg/kg/day)
External			
# of litters examined	12	11	12
# of litters with anomalous fetuses	2	10	2
% of litters with anomalous fetuses	1607	90.9	16.7
Mean values (per litter)			
# of fetuses with variants	0	0.5	0
Incidence of variant (%)	0	14.4	0
# of fetuses with anomalies	0.3	2.7	0.3
Incidence of anomalies (%)	2.4	64.1	4.2
Visceral – Fetal Heads			
# of litters examined	12	9	11
# of litters with anomalous fetuses	0	3	0
% of litters with anomalous fetuses	0	33.3	0
Mean values (per litter)			
# of fetuses with variants	0	0.1	0
Incidence of variant (%)	0	3.7	0
# of fetuses with anomalies	0	0.4	0
Incidence of anomalies (%)	0	16.7	0
Visceral – Torso and Limbs			
# of litters examined	12	11	12
# of litters with anomalous fetuses	0	8	1
% of litters with anomalous fetuses	0	72.7	8.3
Mean values (per litter)			
# of fetuses with variants	0.8	2.9	0.3
Incidence of variant (%)	11.1	63.9	3.6
# of fetuses with anomalies	0	1.5	0.1
Incidence of anomalies (%)	0	38.8	1.0
Skeletal - Skulls			
# of litters examined	12	11	12
# of litters with anomalous fetuses	0	2	0
% of litters with anomalous fetuses	0	18.2	0
Mean values (per litter)			
# of fetuses with variants	0.5	1.6	0.6
Incidence of variant (%)	11.3	60.9	13.0
# of fetuses with anomalies	0	0.2	0
Incidence of anomalies (%)	0	11.4	0
Skeletal – Torso and Limbs			
# of litters examined	12	11	12
# of litters with anomalous fetuses	0	10	1
% of litters with anomalous fetuses	0	90.9	8.3
Mean values (per litter)			
# of fetuses with variants	0.6	3.9	1.0
Incidence of variant (%)	6.9	91.7	12.7
# of fetuses with anomalies	0	2.2	0.1
Incidence of anomalies (%)	0	54.4	2.1

Conclusion:	There were no differences from control in the thalidomide or PI dose groups for maternal, ovarian or uterine data. The thalidomide-treated group exhibited changes consistent with the known teratogenic effect of this compound. The thalidomide group may additionally have had an increase in resorptions and exhibited a possible fetotoxic effect as demonstrated by slightly decreased mean body weights and lengths of the fetuses. There were no effects on any fetal parameters from PI treatment.
Reference:	Burdock, G.A. (1983). Unpublished report no 349-267 entitled "Teratogenicity Study in Rabbits, PI BPA-BI, BPA-DA" dated August 25, 1983 for General Electric Company, Pittsfield, MA; USA from Hazleton Laboratories America, Inc., Vienna, VA, USA Burdock, G.A. (1983). Unpublished report no 349-263 entitled "Two-week Pilot Toxicity Study in Rabbits, BPA-BI, BPA-DA, PI and 4-NPI" dated August 20, 1982 for General Electric Company, Pittsfield, MA; USA from Hazleton Laboratories America, Inc., Vienna, VA, USA
Reliability:	(Klimisch Code 2) Reliable with restrictions. Only one dose level was tested.